On page 7, line 21, delete "theraputic" and insert therefor --therapeutic--.

On page 9, line 19, delete "pannel" and insert therefor --panel--.

On page 10, line 14, delete "are" and insert therefor --is--.

On page 11, line 27, delete "petrie" and insert therefor --petri--.

On page 13, line 2, delete "theraputic" and insert therefor --therapeutic--.

On page 14, line 10, please replace "insotypes" with --isotypes--.

On page 16, line 8, delete "R" and insert therefor --®--.

On page 21, line 23, delete "densigy" and insert therefor --density--.

On page 26, line 6, delete "cytrocentrifuged" and insert therefor --cytocentrifuged--.

On page 35, line 6, delete "stored" and insert therefor --sorted--.

On page 38, line 5, delete "visulization" and insert therefor --visualization--.

On page 39, line 9, delete "the" and insert therefor --The--.

On page 47, line 8, please replace "theraputic" with --therapeutic--.

IN THE CLAIMS

In claim 1, line 3, and in claim 2, lines 1, 3, 13, and 15 please replace the word "normal" with --non-malignant--.

## REMARKS

The Amendment

The specification has been amended to include the patent number of the patent issued on the parent application, which was unknown at the time of filing. The specification has further been amended to correct a series of typographical errors which were identified during prosecution of the parent application and were amended therein. None of the changes made in correcting typographical errors constitute new matter.

Claims 1 and 2 have been amended to more clearly point out that the normal human blood and bone marrow cells with which the monoclonal antibody of the invention does not react are non-malignant human blood and bone marrow cells. This amendment is fully supported in the specification as filed. The cell types which express the MY-10 antigen of this invention are characterized throughout the specification, and in particular, on page 6, the second and third paragraphs. MY-10 antigen is expressed by precursor cells capable of forming granulocytes, monocytes, eosinophils, erythrocytes, and lymphoid cells as well as mixed multi-potent colony-forming cells; the antigen is not found on the mature, disease-free forms of these cells. Further on page 7, last paragraph, the antigen is disclosed to be present on the progenitor cells of a wide variety of human myeloid or lymphoid cells but not on the mature human myeloid or lymphoid cells; the antigen is, however, found on some leukemic cells.

Restriction Under 35 U.S.C. § 121

Restriction has been required between the claims of Group 1, claims 1-10, drawn to a hybridoma and the monoclonal antibody that it produces, Group 2, claims 11-22, drawn to a method of producing purified stem cells, and Group 3, claims 27-30, drawn to a method of medical treatment. Applicant hereby affirms the provisional election to prosecute the invention of Group 1, claims 1-10, without traverse.

The Invention

The subject invention is directed to monoclonal antibodies capable of recognizing an antigen peculiar to human stem cells and to hybridomas producing said monoclonal antibodies. These monoclonal antibodies can be used to select human pluripotent lympho-hematopoietic stem cells from cell populations containing mature cell types and, possibly, malignant cell types, so that the selected stem cells can be used in bone

marrow transplants. One antigen recognized by the monoclonal antibodies of this invention has been designated MY-10 by the inventor, and subsequently designated CD-34 (antibody cluster designation) by the Third International Workshop on Leucocyte Differentiation. (See accompanying "Report on the CD-34 Workshop," from the Proceedings of the Fourth International Conference on Leucocyte Typing, in press.) The MY-10 antigen is expressed by stem cells in the undifferentiated state, but disappears as the cells differentiate and mature. Thus, antibodies to the MY-10 antigen can be used for the identification (and ultimately the separation) of a small number of stem cells among a very large number of mature, differentiated cells in human blood or bone marrow.

Objection and Rejection Under 35 U.S.C. § 112

The specification stands objected to under 35 U.S.C. § 112, first paragraph, as failing to provide adequate description of the invention, based on a requirement for deposit of the hybridoma producing anti-MY-10 monoclonal antibody and of the cell line KG-1a. Claims 3 and 4 stand rejected for the same reasons. These rejections are respectfully traversed.

The hybridoma producing a monoclonal antibody to the antigen designated MY-10 has been deposited with the American Type Culture Collection under accession number HB-8483, as described in the specification on page 8, lines 28-31. The receipt showing that the culture was received by the American Type Culture Collection on January 23, 1984, is enclosed. This deposited hybridoma is cited in issued U.S. Patent 4,714,680, which is offered as evidence of the public availability of the deposit. The undersigned attorney of record, who has authority and control over the conditions of deposit, avers: (a) during the pendency of this application, access to the deposits will be afforded to the Commissioner upon request; (b) all restrictions upon availability of the deposits to

the public will be irrevocably removed upon the granting of a patent on this application; (c) the deposits will be maintained in a public depository for a period of thirty years from the date of deposit or for the enforceable life of a patent or for a period of five years after the date of the most recent request for a sample of the deposits, whichever is longest; and (d) the deposits will be replaced by the Applicant if they should become nonviable or nonreplicable.

The acute myelogenous leukemia cell line, KG-1a, has already been made publicly available by the American Type Culture Collection, Rockville, Maryland, as shown by the enclosed pages from the current ATCC catalog of certified cell lines. Thus, this cell line is publicly available, and any claims dependent on it are enabled independent of need for the applicant to deposit said cell line.

Applicant submits that the access of the public to the specific biological material cited in the specification has been assured by deposits; therefore specification as written provides an enabling disclosure of the invention. Applicant respectfully requests that the rejection of claims 3 and 4 under 35 U.S.C. § 112, first paragraph, be withdrawn.

Claims 1-10 stand rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the disclosure is enabling only for claims limited to the hybridomas and associated anti-MY-10 antibodies used to generate the data of the disclosure. This rejection is respectfully traversed.

The specification provides a number of alternative ways for the ordinary worker to obtain suitable hybridomas, and their associated monoclonal antibodies which are specific for the MY-10 antigen, particularly on pages 8 and 9. The paragraph bridging pages 8 and 9 identifies biological deposits which have the claimed properties. The second paragraph on page 8 provides a method comprising immunizing for the

preparation of hybridomas with cells of the KG-1 or KG-1a cell lines and also provides a screening method for the hybridomas produced. The paragraph beginning on page 9 at line 9, provides a method for purification of the MY-10 antigen which can be used as an alternative immunogen in the method described on page 8, paragraph 2. The last paragraph of page 9 teaches yet another immunogen for use in the method described on page 8, paragraph 2: a cell population which is isolated based on its reactivity with the anti-MY-10 antibody as claimed in U.S. Patent 4,714,680, the parent application. Use of such a cell population as immunogen enables the skilled worker to isolate antibodies to additional stage specific, lineage independent antigens which are present on human pluripotent lympho-hematopoietic stem cells but not present on non-malignant, mature human myeloid and lymphoid cells.

Applicant submits that claims 1-10, drawn to monoclonal antibodies specific for antigens peculiar to human pluripotent lympho-hematopoietic stem cells and the hybridomas which produced them, are fully enabled by the specification, and therefore respectfully request that the rejection of said claims under 35 U.S.C. § 112, first paragraph, be withdrawn.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 1 and 2 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite and vague in their recitation of the term "normal." This rejection is over-come by the present amendment. The term "normal" myeloid and lymphoid cells was used in the claims to contrast the normal, mature cells which do not express the MY-10 with human leukemic cells which may express the MY-10 antigen. The claims have been amended to more clearly point out this distinction. Therefore, Applicant requests that the rejection of claims 1 and 2 under 35 U.S.C. § 112, paragraph 2, be withdrawn.

Rejection Under 35 U.S.C. § 102(a)

The claims stand rejected under 35 U.S.C. § 102(a) as being anticipated by references AR2 (Civin, 1983), AS2 (Civin, 1983), AT2 (Civin, 1983), and AR3 (Strauss, 1983). This rejection is respectfully traversed. These references are all publications by the inventor, published less than one year before filing of the subject application. The accompanying declaration by Dr. Civin describes the activities of the various co-authors in the work covered by these various abstracts. In no case do these activities, as described in Dr. Civin's declaration, rise to the level of an inventive contribution to the subject matter claimed in the subject application. Therefore, applicant respectfully requests that the rejection of the claims under 35 U.S.C. § 102(a) be withdrawn.

Rejection Under 35 U.S.C. § 102(b)

The claims stand rejected under 35 U.S.C. § 102(b) as being anticipated by references AS (Civin, 1982) and AT (Civin, 1982). This rejection is respectfully traversed.

These two abstracts report that antibodies may be elicited by immunization with KG-1a cells. Monoclonal antibodies specific for a variety of antigens were obtained by this procedure. Reference AT did not offer any characterization of these antigens, and thus would not enable one of ordinary skill to recognize which, if any, of the different antigens were peculiar to stem cells. Reference AS reports four monoclonal antibodies that recognize KG-1a cells and further reports the molecular weight of two of them. This characterization would not lead one of ordinary skill to expect that one of these monoclonal antibodies, but not the others, react with an antigen that is peculiar to human stem cells. Therefore, neither abstract enables the skilled worker, without

undue experimentation, to obtain the particular hybridoma that produces an antibody which recognizes an antigen unique to stem cells.

These abstracts do not provide the impetus that would lead one of ordinary skill in the art to select, among the different monoclonal antibodies which may be elicited against KG1a cells, an antibody which recognizes an antigen peculiar only to human pluripotent lympho-hematopoietic stem cells. Therefore, Applicant respectfully requests that the rejection of the claims under 35 U.S.C. § 102(b) as anticipated by references AS and AT be withdrawn.

Rejection Under 35 U.S.C. §§ 102 or 103

Claims 1-10 stand rejected under 35 U.S.C. § 102 as anticipated by, or in the alternative under 35 U.S.C. § 103 as obvious over, references AR4 (Bodger, May, 1983), AS4 (Bodger, 1981) and AT4 (Nadler, 1981). This rejection is respectfully traversed.

The Bodger references teach monoclonal antibodies which are distinct from the monoclonal antibodies of the subject invention because they recognize antigens which are present on cell types not recognized by the antibody of the subject invention. The monoclonal antibodies of this invention are valuable because of their narrow reactivity, which only reaches stem cells and excludes other cell types (non-malignant human myeloid and lymphoid cells). In contrast, cell populations selected by the antibodies disclosed in the Bodger references would contain mature lymphoid cells and monocytes. Therefore the monoclonal antibodies of this invention are distinguished from the antibodies disclosed by Bodger.

In particular, Bodger, 1981, teaches an antibody designated RFB-1. This antibody was tested by the present inventor as described in a Rule 132 declaration submitted in the parent application (a copy of which was submitted in this application); these experiments show that RFB-1 reacts with monocytes and T-cells. Thus, RFB-1 reacts

with both mature human myeloid and lymphoid cells and is outside the scope of the present claims. Bodger (1983) states (on page 1006, last sentence of the paragraph headed "Preparation of Monoclonal Antibodies") that the antibody RFB-HLA-DR reacts with, inter alia, B-cells and monocytes/macrophages. Thus, RFB-HLA-DR antibody also reacts with mature human lymphoid and myeloid cells.

Nadler, et al. reviews a large number of monoclonal antibodies specific for various leukemia cell lines. In Table 11, page 211, the specificity of these antibodies is given in the last column. All of the monoclonal antibodies are reported to be specific for at least one mature myeloid cell type. Another monoclonal antibody discussed by Nadler is J5 (page 215). On page 196 Nadler describes experiments with the J5 antibody and complement to lyse any cells which express the antigen identified by the J5 antibody. In the last four lines of page 196, Nadler reports that the J5 antibody plus complement do not lyse myeloid stem cells; therefore the J5 antibody cannot be the monoclonal antibody of the subject application which does recognizes myeloid stem cells.

Monoclonal antibodies claimed in the subject application do react with human stem cells and do not react with mature human myeloid and lymphoid cells. In contrast, Bodger 1981 and Bodger 1983 teach monoclonal antibodies which react with certain mature human myeloid and lymphoid cells, and they do not teach methods for selecting alternative antibodies which are not reactive with mature human cells. Nadler, et al. teaches two groups of monoclonal antibodies: one which is reactive with mature myeloid cell types, and one which is not reactive with human stem cells. Thus, neither group of antibodies can include the antibodies of the subject application. Nadler teaches methods for selecting monoclonal antibodies which are reactive with antigens present on leukemia cells, but does not teach methods for identifying antigens

peculiar to non-malignant human stem cells. Therefore, following the method of Nadler, et al., would not lead one of ordinary skill in the art to the monoclonal antibodies of the subject invention.

Applicant submits that claims 1-10 are not anticipated by or, in the alternative, obvious over Bodger 1981, Bodger 1983, or Nadler. Therefore, applicant respectfully requests that the rejection of claims 1-10 under 35 U.S.C. § 102 or in the alternative § 103 be withdrawn.

Claims 1-10 stand rejected under 35 U.S.C. § 102(e) as anticipated by, or in the alternative, under 35 U.S.C. § 103 as obvious over U.S. Patents 4,710,457 (DuPont), 4,624,925 (Kung), 4,364,932 (Kung), 4,364,937 (Kung), 4,381,295 (Kung), 4,582,797 (Trowbridge). This rejection is respectfully traversed.

These patents disclose a number of monoclonal antibodies, all of which are reactive with antigens present on mature human lymphoid cells. DuPont teaches a monoclonal antibody recognizing a specific antigen, Leu 200, which is reported to be present on both T and B cells. The four patents to Kung, et al., describe monoclonal antibodies specific for antigens OKT-1, 3, 4, 5, 6, 8, 9, 10 and 11. Monoclonal antibodies specific to these different antigens can be used to distinguish various populations of mature T lymphocytes. Trowbridge discloses a monoclonal antibody specific for antigens T29/33 which is reported (column 2, line 68) to be present on more than 95% of peripheral blood leucocytes. In contrast, the antigen of the subject application is present on less than 5% of peripheral blood leucocytes. Thus, all of the monoclonal antibodies disclosed in these patents react with mature human lymphoid cells. They are therefore distinct from the monoclonal antibody of the subject invention.

Applicant's claimed monoclonal antibodies, which recognize an antigen present on human stem cells but not present on mature human lymphoid cells, are not

anticipated by references which disclose monoclonal antibodies specific for mature human lymphoid cells, nor are they obvious over such references. Therefore, Applicant respectfully request that the rejection of claim 1-10 under 35 U.S.C. § 102(e) or § 103 be withdrawn.

Rejection Under 35 U.S.C. § 103

Claims 1-10 stand rejected under 35 U.S.C. § 103 as being unpatentable over Nadler in view of the known availability of leukemic cell lines such as KG-1 or KG-1a. This rejection is respectfully traversed.

Nadler teaches the selection of monoclonal antibodies specific to antigens found on one or more leukemia cell lines. Nadler teaches the use of these monoclonal antibodies for the characterization and classification of leukemias, as described in the very first paragraph on page 187. This focus is maintained throughout Nadler's review article, up to and including the last sentence on page 218 where the importance of monoclonal antibodies to classify human malignancies is reiterated. While Nadler may impel the researcher in monoclonal antibodies to elicit antibodies specific for a wide range of leukemic cell lines, including KG-1, the screening procedures taught in Nadler for selecting monoclonal antibodies elicited by these cells relate to antigens present on malignant cells rather than antigens which are present on non-malignant human stem cells.

In particular, on page 218, Nadler presents, in Table 13, a list of perceived therapeutic difficulties. Item No. 4 in Table 13 refers to tumor cells which do not possess antigens reactive with the specific monoclonal antibodies selected, with the resultant therapeutic difficulty that such tumor cells could not be attacked by cytotoxic agents coupled to these antibodies or could not be removed by these antibodies in a cleanup procedure which separates leukemic cells from a bone marrow

suspension through binding of malignant cells to their peculiar antibodies. Thus, Nadler teaches one of ordinary skill to select away from an antibody which is not reactive with malignant cells but is specific for human stem cells. In contrast, Applicant has selected an antigen which is not present on most malignant cells specifically for the purpose of selecting non-malignant human stem cells for use in subsequent bone marrow transplants. Therefore, the ordinary worker using the criteria identified by Nadler, selecting for antigens associated with particular tumor lines, would not identify the Applicant's antigen, which is peculiar to non-malignant human stem cells.

Applicant submits that the monoclonal antibodies and hybridomas producing them, as recited in claims 1-10, are not obvious over Nadler, et al. in view of the known availability of leukemia cell lines. Therefore, applicant respectfully request that the rejection of claims 1-10 under 35 U.S.C. § 103 be withdrawn.

Applicant submits that claims 1-10, as amended, are now in condition for allowance and respectfully request prompt allowance of said claims.

Respectfully submitted,

By: _____
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Dated: January 25, 1990
Docket No.: 1107.021635

**ATCC CCL 245 (continued)**
**Species:** Confirmed as human by isoenzyme analysis.
**Erythrocyte Rosette Test:** E, 5%; EA, 5%; EAC, 30%.
**EBNA:** Positive.
**Isoenzymes:** G6PD, B; $PGM_1$, 1; $PGM_3$, 0; ES D, 1; GLO-1, 2.
**Submitted by:** D.W. Golde, School of Medicine, UCLA, Los Angeles, California.
**Prepared and characterized by:** American Type Culture Collection, Rockville, Maryland.

## ATCC CCL 246 KG-1 (Bone marrow, Acute myelogenous leukemia, Human)

**Current medium for propagation:** Iscove's modified Dulbecco's medium, 80%; fetal bovine serum, 20%.

The KG-1 cell line was derived by H.P. Koeffler and D.W. Golde (Science *200:* 1153-1154, 1978). A bone marrow aspirate was obtained from a 59 year-old Caucasian male with erythroleukemia that evolved into acute myelogenous leukemia. The cells were cultured in suspension using $\alpha$ minimum essential medium containing 20% fetal bovine serum and $10^{-4}$ M $\alpha$-thioglycerol. After 24 days in culture the cells were actively proliferating. KG-1 grows predominantly as single cells with numerous small, irregular aggregates of 8 to 14 cells interspersed throughout the static suspension. Morphologically KG-1 cells resemble acute myelogenous leukemia showing considerable pleomorphism with a predominance of myeloblasts and promyelocytes. A small percentage of the cells are mature granulocytes, and occasionally macrophages and eosinophils are also present. The cells stain heavily with ASD chloroacetate esterase and 1-2 percent of the cells stain with peroxidase and Sudan black B.

A unique characteristic of KG-1 cells is their responsiveness to colony-stimulating factor measured by the formation of colonies in soft-agar culture. The KG-1 cells lack specific markers for lymphocytic cells. They have no surface immunoglobulins or Epstein-Barr virus-associated antigens but do express the human Ia-like or DR antigen (Blood *56:* 344-350, 1980). KG-1 differentiates without DNA replication into non-dividing macrophages when exposed to phorbol esters *(ibid., 54:* Suppl. 1, 174a, 1979).

An initial culture designated passage 23 was obtained from Dr. D.W. Golde, UCLA School of Medicine in May 1984.

**DESCRIPTION OF REPOSITORY REFERENCE SEED STOCK**

**Number of Serial Subcultures from Tissue of Origin:** 27.
**Freeze Medium:** Culture medium, 95%; dimethyl sulfoxide (DMSO), 5%; antibiotic-free.
**Viability:** Approximately 85% (dye exclusion).
**Culture Medium:** Iscove's modified Dulbecco's medium, 80%; fetal bovine serum, 20%.
**Growth Characteristics of Thawed Cells:** An inoculum of 2-3 x $10^5$ viable cells/ml in the above culture medium at 37°C results in a doubling time of 48-72 hours over a 5-6 day period provided fresh medium is added at 72-96 hour intervals.
**Plating Efficiency:** The cells cannot be plated.
**Morphology:** Myeloblast-like.
**Karyology:** Chromosome Frequency Distribution 50 Cells: 2n = 46

| Cells: | 1 | 19 | 27 | 2 | 1 |
|---|---|---|---|---|---|
| Chromosomes: | 44 | 46 | 47 | 48 | 49 |

The stemline chromosome number is near-diploid, with the 2S component occurring at 1.8%. Five markers (constitutive markers) were common to most, if not all, metaphases analysed. Modal chromosome number is 47 (or 46 plus a small metacentric chromosome which is smaller than the $G_1$ group chromosome). Normal chromosomes 5, 7, 8, 12 and 17 were monosomic, and others were disomic. The Y chromosome is detected in the Q-banded preparations.
**Sterility:** Tests for mycoplasma, bacteria and fungi were negative.
**Species:** Confirmed as human by isoenzyme analysis.
**Erythrocyte Rosette Test:** E, 0%; EA, 2%; EAC, 0%.
**HLA Antigens:** A30, 31; B35; CW4.
**Surface Immunoglobulins:** Negative.
**EBNA:** Negative.
**Reverse Transcriptase:** Not detected.
**Isoenzymes:** G6PD, B; $PGM_1$, 1-2; $PGM_3$, 0; ES D, 1; Me-2, 1; AK1, 0; GLO-1, 2.
**Submitted by:** D.W. Golde, UCLA, School of Medicine, Los Angeles, California.
**Prepared and characterized by:** American Type Culture Collection, Rockville, Maryland.

## ATCC CCL 246.1 KG-1a (Bone marrow, Acute myelogenous leukemia, Human)

**Current medium for propagation:** Iscove's modified Dulbecco's medium, 80%; fetal bovine serum, 20%.

The variant subline KG-1a of the human acute myelogenous leukemia cell line KG-1 was isolated by H.P. Koeffler, *et al.* (Blood *56:* 265-273, 1980). After the tenth passage, the parental KG-1 cells were cultured in two separate laboratories within the same department under identical conditions. After 35 passages the cells in one laboratory expressed morphological differences from the parent line. The variant KG-1a was composed of undifferentiated promyeloblasts. The cells did not stain for ASD chloroacetate esterase, $\alpha$-naphthyl butyrate esterase or peroxidase.

Both populations exhibit many common characteristics. They share a similar doubling time, are negative for EBNA and VCA, express no surface immunoglobulins and exhibit identical HLA and isoenzyme profiles. In contrast to the parental KG-1 (ATCC CCL 246) the KG-1a population is unresponsive to colony-stimulating factor in soft-agar culture and does not express the Ia-like antigen. KG-1a cells are resistant to phorbol diester induced macrophage differentiation and proliferation of the

cells is unaffected by the presence of phorbol diesters (Blood *62:* 709-721, 1983). The KG-1a cells are morphologically, cytochemically, and functionally less mature than the parental KG-1.

An initial culture designated passage 25 was obtained from Dr. D.W. Golde, UCLA School of Medicine in May 1984.

**DESCRIPTION OF REPOSITORY REFERENCE SEED STOCK**

**Number of Serial Subcultures from Tissue of Origin:** 28.
**Freeze Medium:** Culture medium, 95%; dimethyl sulfoxide (DMSO), 5%; antibiotic-free.
**Viability:** Approximately 80% (dye exclusion).
**Culture Medium:** Iscove's modified Dulbecco's medium, 80%; fetal bovine serum, 20%.
**Growth Characteristics of Thawed Cells:** An inoculum of 2-3 x $10^5$ viable cells/ml in the above culture medium at 37°C results in a doubling time of 46-58 hours over a 6-7 day period provided fresh medium is added at 72-96 hour intervals.
**Plating Efficiency:** The cells cannot be plated.
**Morphology:** Lymphoblast-like.
**Karyology:** Chromosome Frequency Distribution 50 Cells: 2n = 46

| Cells: | 1 | 2 | 3 | 4 | 0 | 4 |
|---|---|---|---|---|---|---|
| Chromosomes: | 43 | 44 | 45 | 46 | 47 | |

The stemline chromosome number is 46 (pseudodiploid), with the 2S component occurring at 5.8%. Seven markers, including five ATCC CCL 246-specific markers, were found in most, if not all metaphases analysed. Another marker ? del (7) was found only in about 50% of the metaphases. Normal chromosomes 5, 7, 8, 12, 17 and 22 were monosomic. The Y chromosome is detected in the Q-banded preparations.
**Sterility:** Tests for mycoplasma, bacteria and fungi were negative.
**Species:** Confirmed as human by isoenzyme analysis.
**Erythrocyte Rosette Test:** E, 0%; EA, 0.5%; EAC, 0%.
**HLA Antigens:** A30, 31; B35; CW4.
**Surface Immunoglobulins:** Negative.
**EBNA:** Negative.
**Reverse Transcriptase:** Not detected.
**Isoenzymes:** G6PD, B; $PGM_1$, 1-2; $PGM_3$, 0; ES D, 1; Me-2, 1; AK1, 0; GLO-1, 2.
**Submitted by:** D.W. Golde, UCLA School of Medicine, Los Angeles, California.
**Prepared and characterized by:** American Type Culture Collection, Rockville, Maryland.

## ATCC CCL 247 HCT 116 (Colon, carcinoma, Human)

**Current medium for propagation:** McCoy's 5a medium, 90%; fetal bovine serum, 10%.

HCT 116 is one of three strains of malignant cells isolated by M. Brattain *et al.* in 1979 from a male patient with colonic carcinoma (Cancer Res. *41:* 1751-1756, 1981). The cells formed colonies in semisolid agarose medium, had a doubling time of 20-22 hours and a saturation density of approximately 4 x $10^5$ cells/$cm^2$. HCT 116 is tumorigenic in athymic nude mice giving rise to epithelioid tumors. Growth of the cells and plating efficiency was enhanced when cultured with feeder layers of murine fibroblasts (J. Nat. Cancer Inst. *69:* 767-771, 1982).

A culture of unknown passage was obtained from M. Brattain in September 1983.

**DESCRIPTION OF REPOSITORY REFERENCE SEED STOCK**

**Number of Serial Subcultures from Tissue of Origin:** +3 at ATCC.
**Freeze Medium:** Culture medium, 95%; dimethyl sulfoxide (DMSO), 5%; antibiotic-free.
**Viability:** Approximately 85% (dye exclusion).
**Culture Medium:** McCoy's 5a medium, 90%; fetal bovine serum, 10%.
**Growth Characteristics of Thawed Cells:** An inoculum of 3 x $10^4$ viable cells/ml in the above culture medium at 37°C will increase 40-fold within 5 days.
**Plating Efficiency:** Approximately 13% in the above culture medium.
**Morphology:** Epithelial-like.
**Karyology:** Chromosome Frequency Distribution 50 Cells: 2n = 46

| Cells: | 2 | 2 | 3 | 1 | 1 | 4 | 1 |
|---|---|---|---|---|---|---|---|
| Chromosomes: | 43 | 44 | 45 | 46 | 47 | | |

The stemline chromosome number is near diploid with the modal number at 45 (62%) and polyploids occurring at 6.8%. The markers, 10q+ and t(?8p;18q) are present in all metaphases and t(9q;?16p-), in 80% of the cells karyotyped. No. 16 is monosomic in the presence of, but disomic in the absence of t(9q;?16p-). Nos. 10 and 18 are monosomic and other chromosomes from those mentioned above are disomic. Q-band observations revealed the presence of the Y chromosome, but not in all cells (50% of cells lacked the Y in G-band karyotypes).
**Sterility:** Tests for mycoplasma, bacteria and fungi were negative.
**Species:** Confirmed as human by isoenzyme analysis (G6PD type B and typical LDH).
**Reverse Transcriptase:** Not detected.

American
Type Culture
Collection

12301 Parklawn Drive, Rockville, Maryland 20852 USA 301-881-2600 Telex ATCCROVE 908-768

*Since 1925*
*an independent non-profit organization incorporated in Washington, D.C. and devoted to the preservation of reference cultures and their distribution to the scientific community*

**AFFILIATED ORGANIZATIONS:**

American Association of Immunologists
American Institute of Biological Sciences
American Phytopathological Society
American Society of Biological Chemists
American Society for Cell Biology
American Society for Microbiology
American Society of Parasitologists
American Society of Zoologists
American Society of Tropical Medicine and Hygiene
Genetics Society of America
Infectious Diseases Society of America
Mycological Society of America
National Research Council-National Academy of Sciences
Society of Protozoologists
Tissue Culture Association

The American Type Culture Collection (ATCC) has received your deposit of a culture in connection with the filing of an application for patent. The following information is provided to fulfill Patent Office requirements.

Name and Address of Depositor: The Johns Hopkins University School of Medicine
Oncology Center 3-121
600 North Wolfe Street
Baltimore, Maryland 21205
Attention: Dr. Curt I. Civin

Date of Receipt of Culture by the ATCC: January 23, 1984

| Scientific Description | Depositor's Reference | ATCC Designation |
|---|---|---|
| Anti-My-10 Hybridoma | (Clone 28/8/8/14/4) | HB 8483 |

The ATCC understands that:

1. The deposit of this culture does not grant to ATCC during the effective term of the patent anticipated a license, either expressed or implied, to infringe the patent, and our release of this culture to others does not grant them a license, either expressed or implied, to infringe the patent.

2. If this culture should die or be destroyed during the effective life of the patent it shall be your responsibility to replace it with a living culture of the same organism. It is also your responsibility to supply sufficient quantity for distribution for the period of time specified.

The ATCC agrees that in consideration for a one-time service charge, not to distribute this culture or any information relating thereto or to its deposit until such time as a patent has been issued disclosing the above deposit except in accordance with U. S. Patent Office Rule of Practice, Rule 14, or until you authorize us to make this strain available. After a patent is issued and we are so informed, the culture will be made available for distribution to the public. The ATCC agrees to maintain the culture for a period of 30 years from the deposit date. We further agree that the name and address of every requestor shall be communicated to the depositor Nonpayment of the service charge within 90 days of the deposit date relieves the ATCC from the above provisions.

The culture was tested and was viable February 1, 1984.

American Type Culture Collection

Date: February 3, 1984

By: Bobbie A. Brandon
(Mrs.) Bobbie A. Brandon, Head
Professional Services Department

cc: Mr. Robert Blackburn, Esq.
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